REMARKS

A. Status of Claims.

Favorable reconsideration of this application as presently amended is respectfully requested. Claims 1 through 7 are pending.

B. Procedural Matters

Applicant notes with thanks the Examiner's acknowledgement that the drawings filed on September 11, 2003 are acceptable.

Applicant notes with thanks the Examiner's acknowledgement of the claim for priority under 35 U.S.C. § 119(a)-(d) or (f).

Applicant notes with thanks the Examiner for the return of forms PTO-1449, filed by Applicant on January 15, 2004, September 30, 2004, and March 27, 2006.

Applicant acknowledges receipt of form PTO-892 listing additional reference(s) considered by the Examiner.

C. Rejections under Section 103

Claims 1 through 7 are rejected under 35 U.S.C. 103(a) as being allegedly unpatentable over Widianto, *et al.* (J. Fragmentation and Bio Engineering, 82(3):199 through 204, 1996, *ref. of record on PTO 1449)* (hereinafter referred to as D1) and Ascenzioni, *et al.* (PLASMID 23: 16 through 26, 1990, *ref. of record on PTO 1449)* (hereinafter referred to as D2). This rejection is respectfully traversed with respect to the claims as currently presented.

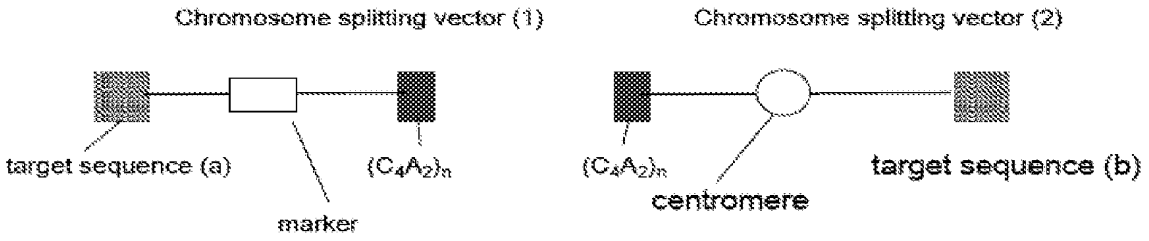
The present invention is directed to a method for modifying a chromosome in a yeast comprising: preparing a linear chromosome splitting vector (1) in an order consisting of a target sequence (a), a marker gene sequence and $(C_4A_2)_n$ sequence (x); preparing a linear chromosome splitting vector (2) in an order consisting of a target sequence (b), a centromere

sequence of a yeast chromosome and $(C_4A_2)_n$ sequence (y) ; and introducing the chromosome splitting vectors (1) and (2) into a yeast, wherein n is each independently an integer of 6 to 10.

As seen from claim 1, one of the features of the present invention is to use a linear chromosome splitting vector (1) in an order consisting of a target sequence (a), a marker gene sequence and $(C_4A_2)_n$ sequence (x) and a linear chromosome splitting vector (2) in an order consisting of a target sequence (b), a centromere sequence of a yeast chromosome and $(C_4A_2)_n$ sequence (y). Other feature of the present invention is to introduce both the splitting vectors (1) and (2) into a yeast and further where n is an integer from 6 to 10.

In the conventional method, it takes a lot of time and efforts to prepare a linear chromosome splitting vector because it takes very long time to prepare a telomere sequence having many repetitive sequences. However, the present invention can provide an efficient and easy preparation of a yeast chromosome splitting vector based on the finding that $(C_4A_2)_n$ sequence (where n is an integer from 6 to 10) that is added to a PCR primer can be multiplied by PCR, and that telomere sequence of yeast can be added to this sequence.

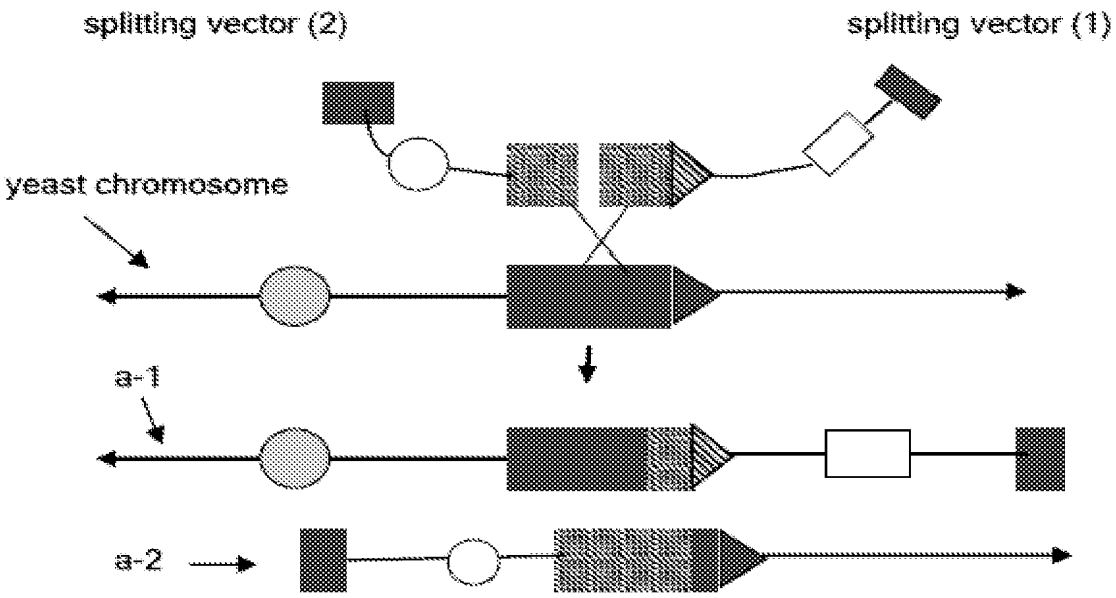
By using both the splitting vectors (1) and (2) simultaneously, splitting and loss of yeast chromosome become possible.



This fact will be explained referring to attached Figures. Figure A is aimed to explain a splitting of chromosome. Figure B is aimed to explain a loss of a part of chromosome.

Turning now to Figure A, an example where both splitting vectors (1) and (2) are used simultaneously so as to split the chromosome at one site. In this case, two chromosome fragments, expressed as a-1 and a-2, respectively, can be generated.

Figure A



The a-1 fragment has a left side portion that contains centromere of the yeast chromosome and a right side portion that is the chromosome replaced by a splitting vector (1). The a-1 fragment has a $(C_4A_2)_n$ sequence at its right end. Since the telomere sequence can be added to the $(C_4A_2)_n$ sequence, the a-1 fragment can be replicated and multiplied.

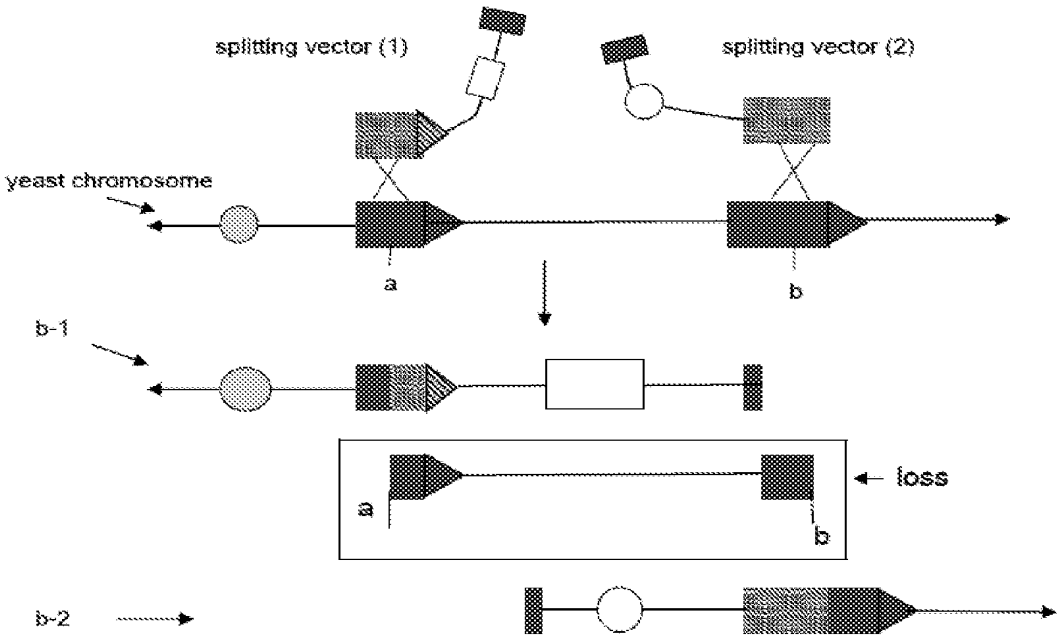
The a-2 fragment has a right side portion of the yeast chromosome and a left side portion that contains centromere of the yeast chromosome which is replaced by a splitting vector (2). This a-2 fragment has a $(C_4A_2)_n$ sequence at its left end. Since the telomere sequence can be added to the $(C_4A_2)_n$ sequence, the a-2 fragment can be replicated and multiplied.

Therefore, when both the splitting vectors (1) and (2) are simultaneously introduced, the yeast chromosome can be split into two fragments and both fragments of the right side portion and the left side portion of the yeast chromosome can be retained. Therefore, yeast

transformed with the splitting vectors (1) and (2) can be survived as far as a gene essential for growth is not split.

Figure B shows an example where both splitting vectors (1) and (2) are used simultaneously so as to split the chromosome at two sites, splitting point a and splitting point b, and thereby loss of chromosome fragment between the splitting points a and b can occur. In this case, as shown in Figure B, b-1 fragment and b-2 fragment are generated, and the chromosome fragment between the splitting points a and b can be lost.

Figure B



Fragment b-1 is generated when recombination of splitting vector (1) has occurred at splitting point a. The fragment b-1 has a left side portion from the left end to splitting point a in which the centromere of the yeast chromosome is contained and a right side portion that is the chromosome replaced by a splitting vector (1). The b-1 fragment has a $(C_4A_2)_n$ sequence at its right end. Since the telomere sequence can be added to the $(C_4A_2)_n$ sequence, the b-1 fragment can be replicated and multiplied.

Fragment b-2 is generated when recombination of splitting vector (2) is occurred at splitting point b. The fragment b-2 has a right side portion from the right end to splitting point b and a left side portion that is replaced by a splitting vector (2). The b-2 fragment has a $(C_4A_2)_n$ sequence at its left end. Since the telomere sequence can be added to the $(C_4A_2)_n$ sequence, the b-2 fragment can be replicated and multiplied.

As a result, the fragment between splitting points a and b is lost. Therefore, yeast transformed with the splitting vectors (1) and (2) can be survived as far as a gene essential for growth is not lost.

As described above, according to the present invention, by using both splitting vectors (1) and (2) simultaneously, an effect can be obtained that a precise analysis of an influence of chromosome splitting on the physiology of yeast cell while the chromosomes are retained in the yeast cell as shown in Figure A.

Further, it becomes possible to make an accurate analysis of effects on, for example, increase of a number of chromosome and variation of a higher structure, which are caused by splitting of chromosome under a condition where all the genes are retained in the cell. Furthermore, as shown in Figure B, it becomes possible to delete a desired portion of a chromosome while maintaining the other portions of the chromosome, which enables a function of deleted potion to be analyzed. As a result, it is expected that a desired substance can be produced efficiently by yeast in which unnecessary genes, for example, energy consuming genes are removed. Furthermore, if a desired region of a chromosome can be transferred into another yeast, the function of yeast chromosome can be analyzed so as to breed useful yeast.

As explained above, in the present invention, it is necessary to a combined use of a linear chromosome splitting vector (1) in an order consisting of a target sequence (a), a marker gene sequence and $(C_4A_2)_n$ sequence (x) and a linear chromosome splitting vector (2) in an order consisting of a target sequence (b), a centromere sequence of a yeast chromosome and $(C_4A_2)_n$ sequence (y).

Turning now to the prior art, D1 and D2 fail to disclose or suggest the linear splitting vectors (1) and (2) and the combination use of the two chromosome splitting vectors.

D1 discloses a method of splitting a yeast chromosome. Plasmids pDW18, pDW10 or pCSV1 disclosed in D1 has two telomere sequences with the opposite direction from each other. The two telomere sequences are joined by BamHI sequence. In the plasmid, stufferDNA(HIS3) is inserted at the BamHI site, that is, between the telomere sequences having opposite directions. This linearized pCSV1 can be used to split yeast chromosome, however, since the plasmids in D1 are conventional vectors, and therefore, it takes a lot of time and efforts to prepare a linear chromosome splitting vector.

For example, to obtain a linear splitting vector for transformation, the plasmid is cleaved with BamHI to delete HIS3 gene, re-circulated, and then, digested with a restriction enzyme at any site of a yeast chromosome portion (target sequence) of the plasmid.[1] As a result, the obtained linear vector comprises in an order consisting of target sequence - telomere sequence (Tr) - telomere sequence (Tr) - cetromere (CEN4) - marker gene (URA3) - target sequence. The obtained linear vector of D1 has target sequences at both ends. Since these target sequences of the both ends are obtained by a digestion of the yeast chromosome, the vectors of D1 just cause to split the yeast chromosome. Further, D1 does not disclose or suggest that a sequence in the linear vector of D1 corresponds to splitting vectors (1) and (2).

Moreover, the linear vector of D1 contains two telomere sequences that are joined with the opposite direction to each other. Thus, the structure of the linear vector of D1 is different from that of linear splitting vectors (1) and (2) of the present invention. That is, D1 fails to disclose or suggest a linear vector having a target sequence at one end and telomere sequence at the other end. Furthermore, D1 fails to disclose or suggest the combined use of linear vectors (1) and (2) and the essentiality of combination thereof.

As stated above, very complicated process is necessary to obtain linear splitting vector from teaching of D1. This pCSV1 of D1 is described as a conventional vector that requires a complicated process to obtain linear vector from pCSV1.[2] Contrary to pCSV1, the

[1] See page 201, left column lines 10 to 20, and right column, lines 20 to 27 of D1.
[2] See page 3, line 8 to page 4, line 5 of D1.

linear chromosome splitting vectors (1) and (2) of the present invention can be prepared very easily.

D2 is a reference that describes a function of a plant or animal gene introduced in yeast (gene in a YAC library). In D2, it is examined whether or not yeast ARS gene adjacent to telomere sequence can act as an ARS. In D2, it is described that yeast ARS gene adjacent to telomere sequence was removed, and C_4A_2 sequence, which is a synthetic telomere sequence, was added to the removed portion to obtain a linear vector. The obtained linear vector was used to transform the yeast. D2 described that the 15 to 40% of the length of the linear vector was decreased from the end to which synthetic telomere sequence was added, but it did not cause any change to yeast chromosome and YAC portion.[3]

The linear vector of D2 has a telomere sequence at one end and C_4A_2 sequence at the other end.[4] That is, the linear vector of D2 has a telomere sequence - marker gene (LEU2) - ARS (CEN) - C_4A_2 sequence. Thus, the sequence of D2 is completely different from that of the splitting vectors (1) and (2). Moreover, linear vector of D2 does not have target sequence. D2 does not disclose or suggest the sequences that correspond to the splitting vectors (1) and (2) used in the present invention. Thus, the experiment of D2 was performed merely in order to confirm the function of telomere sequence, but not for the purpose of split or loss of the chromosome. In this way, D2 fails to describe or teach splitting vectors (1) and (2). D2 fails to disclose or suggest the combined use of linear vectors (1) and (2) and the essentiality of combination thereof.

As stated above, D1 and D2 fail to disclose or suggest the sequence of the linear splitting vectors (1) and (2) of the present invention. Furthermore, D1 and D2 fail to disclose or suggest that the combined use of linear splitting vectors (1) and (2) is essential.

D1 discloses a linear vector having target sequences at both ends of the vector and telomere sequences at a middle portion of the chromosome, in which the telomere sequences has opposite direction. On the other hand, D2 discloses a linear vector having a telomere

[3] See page 25, left column, lines 33 to 38 of D2.
[4] See page 16, lines 1 to 3 of the abstract of D2.

sequence at one end and a C_4A_2 sequence at the other end. Since the purposes of D1 and D2 are quite different from each other, the sequences of the both ends of D1 and D2 are different.

Therefore, there is no motivation to combine D1 and D2. Even if the D1 and D2 are combined, and C_4A_2 sequence is used instead of telomere sequence of D1, the obtained sequence has C_4A_2 sequence at a middle portion of the chromosome and has target sequence at both ends. Therefore, it is not possible to obtain splitting vectors (1) and (2) even if D1 and D2 are combined. Furthermore, splitting vector of D1 is only splits the chromosome.

In this way, the simultaneous use of both splitting vectors (1) and (2) of the present invention would not have been obvious over D1 and D2. Further, it would not have been obvious to even those skilled in the art to obtain a superior effects as stated above by using of both splitting vectors (1) and (2) simultaneously as claimed in the present invention.

The dependent claims incorporate all of the subject matter of their respective independent claims and add additional subject matter which makes them *a fortiori* independently patentable over the art of record. Accordingly, Applicants respectfully request that the outstanding rejections of the dependent claims be reconsidered and withdrawn.

D. CONCLUSION

In view of the foregoing, it is respectfully submitted that this application is in condition for allowance, and favorable action is respectfully solicited.

If the Examiner has any questions or concerns regarding the present response, the Examiner is invited to contact Ajay A. Jagtiani at 703-591-2664, Ext. 2001.

The Commission is hereby authorized by this paper to charge any fees during the entire pendency of this application including fees due under 37 C.F.R. §§ 1.16 and 1.17 which may be required, including any required extension of time fees, or credit any overpayment to Deposit Account 10-0233-NANJ-0009-US1.

Respectfully submitted,

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